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# S14 Terminal Oxidases

## 14L1

### Proton transfer in NO-reducing heme-copper oxidases

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Heme-copper oxidases (HCuOs) terminate the respiratory chain in mitochondria and most bacteria. They are integral membrane proteins that catalyse the reduction of oxygen, and they convert the liberated free energy into a proton-motive force across the membrane. The HCuO superfamily has been divided into the oxygen-reducing A-, B- and C-type oxidases as well as the bacterial NO reductases (NOR), where the latter are not $O_2$-reducers but instead catalyse the reduction of NO in the denitrification process. Proton transfer to the catalytic site in the mitochondrial-like A family occurs through two well-defined pathways termed the D- and K-pathways. The B, C, and NOR families differ in the pathways as well as the mechanisms for proton transfer to the active site and across the membrane. In the NORs, recent structural work has shown that there are possible differences in the proton transfer (PT) pathways between the NOR that uses cyt. *c* as electron donor (*c*NOR) and NOR that uses quinol (qNOR), where qNOR has a putative PT pathway from the cytoplasm, in contrast to *c*NOR which is known to be non-electrogenic and use periplasmic protons for NO-reduction ($2NO + 2e^- + 2H^+ \rightarrow N_2O + H_2O$). We study the PT pathways and mechanisms specifically in the C-type HCuO as well as both *c*NOR and qNOR using site-directed mutagenesis in combination with time-resolved spectroscopy during single turnovers, and recent results from these studies will be presented and discussed.

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## 14L2

### Functions of the hydrophilic channels of mitochondrial forms of cytochrome oxidase from studies of yeast mutants

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Cytochrome *c* oxidase (CcO) catalyses electron transfer from cytochrome *c* to molecular oxygen in a manner that is coupled to transmembrane proton transfer. Mammalian mitochondrial CcO is a dimer with 13 different polypeptides in each monomer. Subunits I, II and III form its catalytic core and structurally similar homologues of these three subunits are present in the simpler 3–4 subunit bacterial CcOs. The roles of the additional non-core supernumerary subunits of mammalian CcOs are not well understood. Furthermore, despite the structural similarities of the mammalian and bacterial core subunits, difficulties in reconciling existing experimental data preclude a definitive resolution of whether their coupling mechanisms are the same. In particular, a hydrophilic H channel, first identified in the bovine CcO structure [1], has been proposed to be the route for its translocated protons. This contrasts with the view, supported by extensive mutagenesis work, that the D channel fulfils this function in bacterial CcOs. However, an alternative possibility is that the H channel, which is weaker and discontinuous in bacterial CcOs, is functionally required in all forms of CcO but acts instead as a 'dielectric channel' [2] to enable energetically facile transient electron transfer through buried haem *a*. If it does indeed act in this manner, it is further possible that this structure in mitochondrial CcOs may provide, in conjunction with nearby supernumerary subunits, a means of control of core enzymatic function. Evidence for these proposals will be reviewed, together with the new data being obtained with mutant forms of yeast (*Saccharomyces cerevisiae*) CcO [3].

#### References

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## 14L3

### Crystal structure of the alternative oxidases: New insights into the catalytic cycle

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